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Amy Rosenberg
Barthélemy Demeule
Editors

Biobetters

Protein Engineering to Approach the Curative

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Protein Engineering to Approach the Curative

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Preface

> …and what never frees us from the cost of knowledge, which is to act on what we know again and again
>
> Marge Piercy, American poet

Curative or preventive therapies for human and animal diseases would provide optimal benefit, not only to individual patients in terms of quality and quantity of life but as well to the healthcare system in minimizing the need for chronic and often very expensive treatments and supportive measures. Preventive therapies in the form of vaccines have been termed our "most effective public health measures,[1]" yet their development provides limited economic incentive. Other preventive therapies may be restricted to small subsets of patients who have a relatively high probability of manifesting disease, such as prophylactic mastectomy/oophorectomy in patients with known high-risk mutations for breast and ovarian cancers (e.g., BRCA mutation positive patients). While these measures are critically important to the patients, the savings to the healthcare system are much more modest. In contrast to therapeutic proteins such as monoclonal antibodies that address peripheral mediators of disease (e.g., TNF inhibitors), curative therapies, aimed at the underlying pathophysiology of the disease (e.g., RA), remain elusive despite renewed emphasis.[2] These unfortunate circumstances highlight the need for optimal, not just effective, therapeutics to bridge the gap between an effective but expensive and chronically administered therapeutic and a curative therapy for diverse clinical entities. Thus, while the approval of a new therapeutic protein for an unmet clinical need is always an important advance, once approved, there appears to be limited impetus to improve the clinical performance of the initial product even though the means and ways to do so may be known and feasible. The advent of "biosimilars" and their economic impact (captured in the chapter by Berndt et al.) will hopefully change this landscape and offer strong economic incentive for development of biobetters.

[1] Bulletin of the World Health Organization, Volume 86 Number 2, February 2008, 81–160

[2] http://www.ncats.nih.gov/funding-and-notices/can/can.html

This book was conceived to address the bridge to curative therapies, to improve upon the gains of current therapeutics by enhancing their efficacy and safety pending the development of curative therapies. We have focused on two types of therapeutic proteins as providing illustrations for important concepts pertaining to biobetters: monoclonal antibodies, the most rapidly growing class of therapeutic proteins, and therapeutic enzymes for lysosomal storage diseases, therapeutics which are frequently the sole treatments for rare and often rapidly fatal "inborn errors of metabolism."

The means to enhance pharmacokinetics (PK), to prevent degradation in the in vivo environment, to minimize immunogenicity, and to enhance product efficacy without incurring novel safety issues are common aspirations for both types of therapeutic proteins. While enhancements in PK by technologies such as pegylation are important improvements, especially for maximizing dosing interval, and thus important for patient quality of life, the gains to be accrued from more highly effective therapies, both to the patient and to the healthcare system, are the key focus of this book. Thus, the chapters regarding the means to enhance efficacy, such as improved targeting to critical target tissues (e.g., penetrating the blood–brain barrier in the absence of inflammation; targeting muscle with its low expression of critical receptors for therapeutic enzymes), minimizing immunogenicity via protein engineering or tolerance induction regimens, optimizing affinity of mAbs for receptors or improving effector function via engineering of CDR and Fc regions, respectively, or via employment of novel scaffolds, are of key importance.

Of course, great caution is warranted in such undertakings, as it may be possible to be too "biobetter." For example, sustained activity of some therapeutic protein hormones may have unintended outcomes if downstream mediators which are key factors in induction/proliferation of malignancies are induced and sustained at high levels rather than having a transient exposure profile. For enzyme deficiency disorders, sustained prolonged activity of the therapeutic enzyme leading to efficient and near complete substrate depletion has the potential to cause serious problems. For example, in the setting of Gaucher Disease, an overly efficient enzyme replacement therapy (ERT) could cause rapid conversion of glucocerebrosides to ceramides, high levels of which may cause cellular apoptosis. Similarly, in phenylketonuria (PKU), there is concern that an overly active phenylalanine lyase could drop phenylalanine to such low levels that protein production may potentially be limited in younger children and those with metabolic stress. As to monoclonal antibodies and their derivatives, the design of biobetters should take into account the fact that higher affinities do not necessarily translate to clinical benefit and that new constructs targeting multiple pathways (e.g., bispecifics) should be carefully evaluated for their potential to generate unintended adverse effects. Use of preclinical animal models of diseases as well as carefully conducted clinical studies to test more highly active biobetters should mitigate against the specter of "too biobetter."

We would like to conclude with a reminder of addressing great efforts from a great President facing a monumental task, that of President John F. Kennedy in considering a program to put a human on the moon. In considering the magnitude of the effort, he said, "We choose to go to the moon. We choose to go to the moon

in this decade and do the other things, not because they are easy, but because they are hard, because that goal will serve to organize and measure the best of our energies and skills, because that challenge is one that we are willing to accept, one we are unwilling to postpone, and one which we intend to win…." Unlike putting a human on the moon, we already have the ways and means and knowledge to optimize our therapeutic protein products. So let us too proceed to utilize the best of our energies, skills, and knowledge to improve therapeutic proteins to optimize clinical outcomes for suffering patients.

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Dr. Amy Rosenberg received her M.D. from Albert Einstein College of Medicine and trained in internal medicine and infectious diseases. She was a postdoctoral fellow in Al Singer's Laboratory in the NCI before coming to CBER, FDA. She is the director of the Division of Biotechnology Review and Research III, Office of Biotechnology Products in CDER, FDA which regulates diverse protein therapeutics, including monoclonal antibodies, fusion proteins, enzyme replacement therapies, hematologic and somatic cell growth factors, and immunomodulatory agents. Her particular interest is in immune tolerance induction in diverse clinical settings including autoimmunity, therapeutic protein immunogenicity, and transplantation.

Dr. Barthélemy Demeule obtained his Ph.D. at the University of Geneva, Switzerland, where he started his investigations on the physicochemical stability of biopharmaceuticals. After a postdoctoral work at Genentech, Inc. focused on the effect of the in vivo environment on antibody–antigen interactions, he stayed in the company where he held positions of increasing responsibilities. He currently leads a group of scientists responsible for the pharmaceutical development of monoclonal antibodies in the last phases of clinical development. He also serves on the editorial board of the *European Journal of Pharmaceutics and Biopharmaceutics*.

Part I
Therapeutic Enzymes

Targeting Glucocerebrosidase to Macrophages for Effective Treatment of Patients with Gaucher Disease: Setting the Paradigm of a "Fit for Purpose" Approach to Enzyme Replacement Therapy

Roscoe O. Brady

Gaucher disease is one of the most prevalent hereditary metabolic storage disorders of humans. A patient with an enlarged spleen was described by the French medical student Phillipe C. E. Gaucher who thought she had a splenic neoplasm (Gaucher 1882). Brill (1901) suggested that patients with such a presentation represented a familial disorder. It was reported that the spleen of these patients contained a hyaline-like material (Marchand 1907). Lieb (1924) believed that the accumulating material in the spleen was galactocerebroside. However, the optical rotation of the sugar released by acid hydrolysis was inconsistent with this assumption. Aghion (1934) demonstrated that glucocerebroside was the substance that accumulated (Fig. 1a). The kinetics of the formation of glucocerebroside was found to be normal in patients with Gaucher disease (Trams and Brady 1960). It was postulated that the metabolic defect in these patients was of a catabolic nature. Several years later, an enzyme was discovered in mammalian organs that catalyzed the hydrolytic cleavage of glucose from glucocerebroside (Brady et al. 1965a) (Fig. 1b). Reduced activity of this enzyme was shown to be the cause of Gaucher disease (Brady et al. 1965b, 1966). The possibility of overcoming the insufficient glucocerebrosidase by enzyme replacement was proposed (Brady 1966).

I wished to obtain a human source of glucocerebrosidase if it were possible. One evening it occurred to me that the placenta might be useful in this regard. The next day I homogenized some fresh placental tissue and found that it did indeed contain glucocerebrosidase. My colleagues and I were able to obtain small amounts of comparatively pure glucocerebrosidase from this tissue (Pentchev et al. 1973). When we injected it into two patients with Gaucher disease, we found a significant decrease in the quantity of glucocerebroside that had accumulated in the liver (Brady et al. 1974). Moreover, there was marked decrease of the elevated glucocerebroside that

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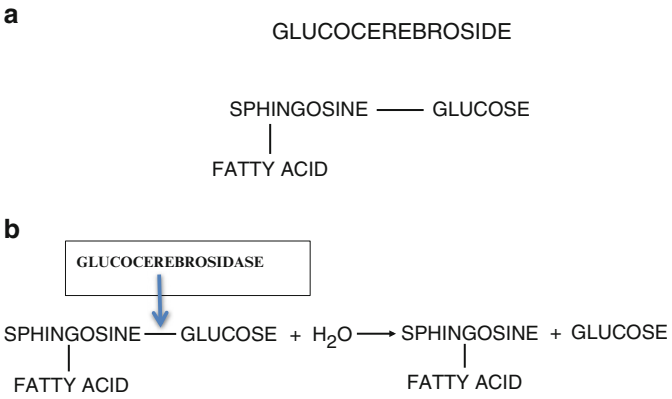
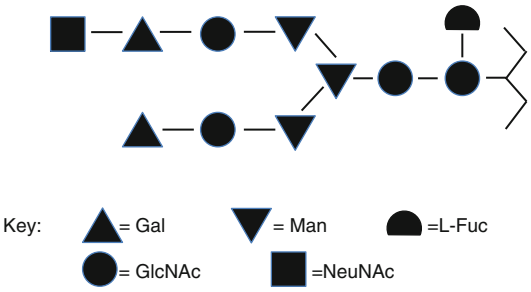


Fig. 1 (**a**) Accumulating material in Gaucher Disease. (**b**) The catabolism of glucocerebroside is initiated by the enzyme glucocerebrosidase

was associated with red blood cells in the circulation (Brady et al. 1974). Of particular interest was the lengthy period of time following the injection of glucocerebrosidase before glucocerebroside associated with red blood cells rose toward pre-injection levels (Pentchev et al. 1975).

We were quite surprised when next patient we treated with glucocerebrosidase showed an insignificant clearance of glucocerebroside. We discovered that she had accumulated 24 times the quantity of glucocerebroside in her liver than the first recipient and 11 times more than the second. We realized we would have to improve our purification procedure in order to obtain sufficient quantities of glucocerebrosidase to treat such patients. We achieved this goal by developing a technique to isolate the enzyme based on the incorporation of two hydrophobic column chromatography steps in the purification procedure (Furbish et al. 1977). We were quite startled with the findings when we injected enzyme purified in this fashion into seven patients with Gaucher disease. Three of the patients had significant reductions of glucocerebrosidase but four showed no change at all. This was not caused by any lack of catalytic activity of the preparation. Glucocerebroside specifically accumulates in macrophages (Kupffer cells) in the liver. We suspected that we probably were not *targeting* the glucocerebrosidase to macrophages that are involved in biodegrading sphingolipids arising from rapidly turning over cells such as white and red blood cells and blood platelets. We felt that the inability to deliver glucocerebrosidase to macrophages was caused by the requirement to treat the placental extract with butanol in order to remove lipids that prevented the binding of glucocerebrosidase to the hydrophobic columns. Among the lipids that were extracted by this method was phosphatidylserine that had two specific effects on glucocerebrosidase. It was shown by Dale et al. (1976) and Choy (1984) that it markedly stimulated the activity of this enzyme. Moreover, Schroit et al. (1984)



Abbreviations: Gal = galactose; Man = mannose; L-Fuc = fucose; GlcNAc = N-acetylglucosamine; NeuNAc = N-acetylneuraminic acid

Fig. 2 Carbohydrate unit of native glucocerebrosidase

discovered that phosphatidylserine was specifically recognized by macrophages. We tried to re-lipidate glucocerebrosidase that had been purified by hydrophobic column chromatography with phosphatidylserine but achieved only modest success in increasing its delivery to macrophages in which glucocerebroside accumulates.

Thus, my associates and I embarked on a different approach to target glucocerebrosidase to macrophages. It was known that macrophages have a lectin (carbohydrate binding protein) on their surface that has a high affinity for mannose-terminal oligosaccharides (Stahl et al. 1978). Glucocerebrosidase is a glycoprotein with four oligosaccharide side chains, three of which have a complex array of sugars terminating with *N*-acetylneuraminic acid that shields three underlying mannose residues (Takasaki et al. 1984) (Fig. 2). A series of investigations was undertaken to determine whether altering the oligosaccharide chains of glucocerebrosidase to expose such mannose residues would affect the cellular uptake of the enzyme. We sequentially removed the three external moieties of the oligosaccharide chains with exoglycosidases (Fig. 3) producing the mannose-terminal glycoform of glucocerebrosidase (Fig. 4) (Furbish et al. 1978, 1981; Steer et al 1978; Brady and Furbish 1982). We discovered that mannose-terminated glucocerebrosidase was taken up by macrophages 50 times more effectively than native placental glucocerebrosidase. We began to administer glucocerebrosidase modified in this fashion intravenously to patients with Gaucher disease. The first trial consisted of seven adults and one child with Gaucher disease. Only the child showed evidence of benefit (Barton et al 1990). We realized that we should have carried out a dose–response study before such a trial. We therefore undertook that investigation and found that a consistent reduction of accumulated glucocerebroside was obtained by administering 60 IU of mannose-terminal glucocerebrosidase per kg of body weight. An investigation of this amount of enzyme administered to 12 patients with Gaucher disease revealed

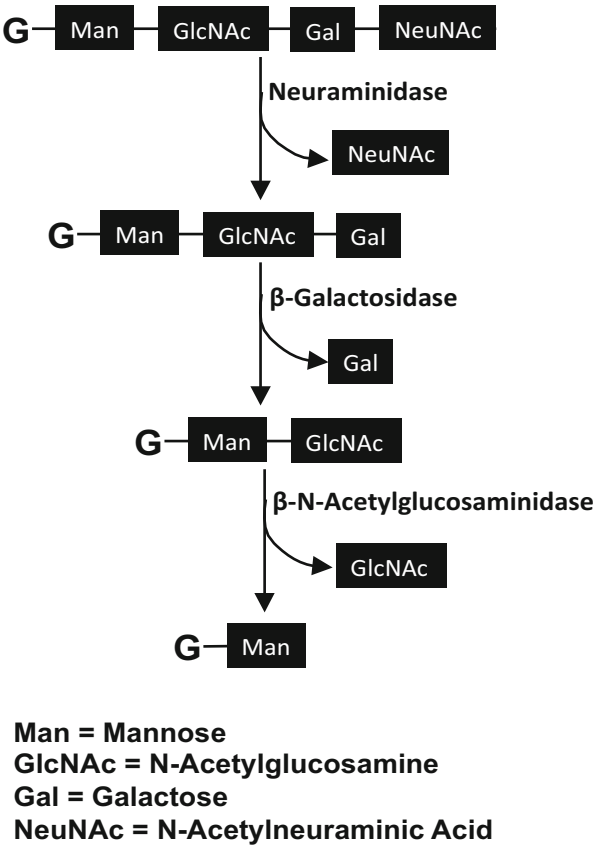


Fig. 3 Enzymatic modification of glucocerebrosidase (G)

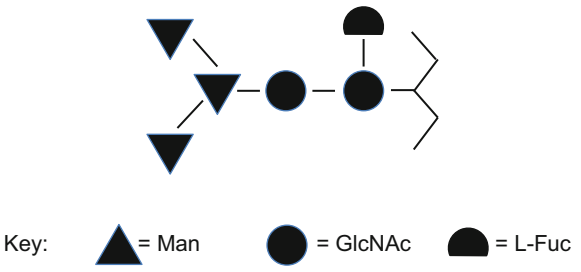


Fig. 4 Carbohydrate unit of mannose terminated glucocerebrosidase

highly beneficial responses in all recipients. There was a reduction of the size of the enlarged liver and spleen, an increase in blood platelets, an increase in hemoglobin and improvement of the skeleton in all recipients (Barton et al. 1991). Based on these findings, enzyme replacement therapy was approved by the U.S. Food and Drug Administration (FDA) for patients with Gaucher disease on April 5, 1991. It was quickly realized that the collection and processing of sufficient placentas to treat all of the patients with Gaucher disease who required this therapy would be extremely difficult, if not impossible. The Genzyme Corporation decided to produce the enzyme by recombinant technology in Chinese hamster ovary cells in large bioreactors. The oligosaccharide side chains of glucocerebrosidase obtained in this process also required modificationin the same manner as the placental glucocerebrosidase. Recombinant glucocerebrosidase was approved for the treatment of patients with Gaucher disease by the U.S. FDA in 1994. It was shown to be as effective as oligosaccharide-modified placental glucocerebrosidase (Grabowski et al. 1995).

More than 6,000 patients with Gaucher disease throughout the world are now being successfully treated with macrophage-targeted glucocerebrosidase. Other hereditary metabolic disorders affect different target tissues bearing different lectins and thus, will require related procedures to deliver a therapeutic protein to treat those conditions effectively. Alternatively, genetic regulation of the activity of the glycotransfereases involved in creating the glycoforms of required enzymes may evolve as a useful strategy to obtain effective products.

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Challenges of Enzyme Replacement Therapy: Poor Tissue Distribution in Lysosomal Diseases Using Pompe Disease as a Model

Priya S. Kishnani

After over two decades of concept studies, animal model studies, and safety trials, the FDA approved the first human enzyme replacement therapy (ERT) for Gaucher disease type I, a lysosomal storage disease (LSD), in 1991 (Barton et al. 1991). The therapeutic enzyme effectively lowered buildup of glycosylceramide in the liver, spleen, and bone marrow, among other tissues, resulting in notable clinical improvements including reduced organomegaly, and improvements in hematologic, and skeletal parameters. This landmark achievement marked not only the progress towards reaching a life-saving treatment for Gaucher disease, one of the most common LSDs, but also planted the seeds of hope that ERT could be utilized for the other LSDs. To date, seven LSDs are being treated with ERT including Mucopolysaccharidosis I (MPS I), MPS II, MPS IV, MPS VI, Gaucher disease, Fabry disease, and Pompe disease. For Gaucher disease there are three different ERTs currently approved and two oral medications that reduce substrate accumulation. For Fabry disease there are two approved ERTs. Several other disease-specific ERTs are currently in development (Table 1).

ERT has revolutionized treatment for patients with LSDs, dramatically improving lifespan, increasing overall quality of life, and diminishing the extent of organ involvement. Despite this progress, certain challenges have been identified in patients receiving ERT due to a number of factors including the following: minimal or no enzyme delivery to all necessary target sites; delay in diagnosis enabling substrate buildup with often irreversible consequences prior to the start of ERT; inability of the therapeutic enzyme to reach certain sanctuary sites, including the central nervous

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Table 1 Overview of therapies either FDA approved or in commercial development for LSDs

Lysosomal storage disease	Deficient enzyme	Current therapy trade name (recombinant enzyme)	FDA/EMA approval or clinical trial status	Clinical improvements	Limitations of current therapy (persistent issues)
Gaucher disease, Type I	Acid beta-glucosidase	ERT	FDA 1991; EMA 1997	Reduction of organomegaly, hematological improvements, improvement in BMD, reduction of bone pain, improved quality of life (Anderson et al. 2014)	Osteopenia, pulmonary infiltrates, mesenteric lymphadenopathy, Gaucheromas (Gaucher-cell pseudo tumors), Parkinson's-like symptoms, pulmonary hypertension, no correction of CNS disease (Poll and Vom Dahl 2009)
		Cerezyme (imiglucerase)			
		Zavesca (miglustat)	EMA 2002; FDA 2003		
		VPRIV (velaglucerase alfa)	FDA 2010; EMA 2010		
		Elelyso (taliglucerase alfa)	FDA 2012		
		SRT	FDA 2014		
		Cerdelga (eliglustat)			
Fabry disease, classic, late-onset	Alpha-galactosidase A	ERT	EMA 2001; FDA 2003	Improvements in neuropathic pain, improved nephropathy, improved cardiomyopathy	Cardiac fibrosis, arrhythmia risk, stroke risk, kidney failure, white matter changes in brain (Pieroni et al. 2013), increase in antienzyme antibodies
		Fabrazyme (agalsidase beta)			
		Replagal (agalsidase alfa)	EMA 2001		
Mucopolysaccharidosis I, Hurler-Sheie spectrum	Alpha-L-iduronidase	ERT	FDA 2003; EMA 2003	Stabilization or improvement of joint range of motion, sleep apnea, and left ventricular hypertrophy (Clarke et al. 2009)	Neurological, cardiac, ophthalmologic (corneal clouding) and skeletal complications remain (Clarke et al. 2009); antienzyme antibodies
		Aldurazyme (laronidase)			
		Hematopoietic stem cell transplant			

Lysosomal storage disease	Deficient enzyme	Current therapy trade name (recombinant enzyme)	FDA/EMA approval or clinical trial status	Clinical improvements	Limitations of current therapy (persistent issues)
Mucopolysaccharidosis VI	*N*-Acetylgalactosamine-4-sulfatase	ERT Naglazyme (galsulfase)	FDA 2005; EMA 2006	Improvements in gait, respiratory ability and general quality of life (Valayannopoulos and Wijburg 2011)	Opthalmologic and skeletal complications remain
Mucopolysaccharidosis II, severe and attenuated	Iduronate-2-sulfatase	ERT Elaprase (idursulfase)	FDA 2006; EMA 2007	Improvements in gait, decreased liver and spleen volumes, reduced urinary GAG levels (Muenzer et al. 2006)	Neurological and skeletal complications remain (Muenzer et al. 2006)
Pompe disease	Acid alpha-glucosidase	ERT	FDA 2006	Improved motor function, respiratory function and cardiac function	Musculoskeletal damage and progression despite ERT, anterior horn cell disease and bulbar involvement
Infantile		Myozyme (aglucosidase alfa)			
Late-onset		Lumizyme (aglucosidase alfa)	FDA 2010		Cardiac arrhythmias, fibrosis, antienzyme antibodies
Mucopolysaccharidosis IV, type A	*N*-Acetylgalactosamine-6-sulfatase	ERT Vimizin (elosulfase alfa)	FDA 2014	Decrease in urinary keratin sulfate, increase in walk distance and improvement in stair climbing overall	Skeletal complications remain, significant morbidity

(continued)

Table 1 (continued)

Lysosomal storage disease	Deficient enzyme	Current therapy trade name (recombinant enzyme)	FDA/EMA approval or clinical trial status	Clinical improvements	Limitations of current therapy (persistent issues)
Lysosomal acid lipase deficiency, CESD and Wolman disease	Lysosomal acid lipase	ERT Sebilipase alfa	For CESD, phase 1 completed in 2012; For Wolman disease, phase 2/3	Successful completion of Phase 3 tral	Disease progression remains
Alpha-mannosidosis	Alpha-mannosidase	ERT Lamazym (alpha-mannosidase)	Phase 3	Urinary and cerebrospinal fluid oligosaccharides decreased, motor function improved	Low permeability of the blood–brain barrier
Niemann Pick, type B	Acid sphingomyelinase	ERT Acid sphingomyelinase	Phase 2 trial	Drug well tolerated at lower doses, but at doses of 0.6 and 1.0 mg/kg the cytokine and bilirubin levels were elevated	Progression of lung disease
Mucopolysaccharidosis VII	Beta-glucuronidase	ERT UX003	Phase 1/2 enrolled in 2013	Improvements in walking capabilities, respiratory ability, and general quality of life (Valayannopoulos and Wijburg 2011)	Neurological and skeletal complications remain
Metachromatic leukodystrophy and Krabbe disease	Arylsulfatase A and galactocerebroside β-galactosidase, respectively	ERT HSCT	N/A	Stalls disease progression	Limitation of ERT is that intravenously delivered enzymes generally do not cross the blood–brain barrier
Niemann Pick, type A	Acid sphingomyelinase	ERT	N/A	N/A	Severe neurovisceral form, poor prognosis and limited survival

system (CNS), bone, and cartilage (Hollak and Wijburg 2014); and immune responses to the exogenous enzyme abrogating its effectiveness, especially in patients who are cross-reactive immunologic material (CRIM) negative.

These therapeutic deficiencies are evidenced in varying ways in the different LSDs. In Gaucher disease, patients receiving ERT can still experience osteopenia, pulmonary infiltrates, mesenteric lymphadenopathy, Gaucheromas (Gaucher-cell pseudo tumors), seizures, Parkinson-like symptoms, and pulmonary hypertension (Bennett and Mohan 2013; Poll and Vom Dahl 2009). An increased incidence of multiple myeloma is also present, which is believed to be caused by elevated levels of IL-6 causing clonal expansion of B cells (Rosenbloom et al. 2005). ERT, unable to cross the blood–brain barrier, fails to halt neurologic symptoms in the neuronopathic subtype of Gaucher disease (Schiffmann et al. 2008), MPS I, and MPS II (Muenzer 2014). In Fabry disease, an LSD caused by a deficiency of the enzyme alpha-galactosidase A resulting in glycosphingolipid buildup in vascular endothelium, ERT with agalsidase alfa reduces and/or stabilizes symptoms of neuropathic pain, nephropathy, and cardiomyopathy (Eng et al. 2001). Nonetheless, even with ERT, patients with Fabry disease continue to experience complications such as renal failure, strokes, arrhythmias, proteinuria, chronic neuropathic pain, and myocardial fibrosis. Likely contributing to therapeutic failure is inefficient delivery to certain target sites, and development of antibodies with neutralizing activity to ERT in male patients with Fabry disease (Linthorst et al. 2004). Finally, myocardial fibrosis, a common finding in older patients with Fabry disease, may be present prior to treatment initiation and creates a hurdle, sometimes a barrier, to the efficacy of exogenous enzyme (Weidemann et al. 2013, 2014).

The mucopolysaccharidoses, another subgroup of LSDs, have also encountered both successes and shortcomings through ERT (Muenzer 2014). In patients with MPS I, II, and IV, complications of the heart, skeletal system, lungs, and gastrointestinal tract (organomegaly, hernias) visibly improve with ERT (Noh and Lee 2014). At the same time, several symptoms tend to persist with ERT, including cardiac valve disease (stagnation of mitral/aortic valve stenosis; progressive aortic valve regurgitation), skeletal/joint disease (dysostosis multiplex), and airway disease (Muenzer 2014) likely attributable to failure of ERT to penetrate such tissues sufficiently. As in neuronopathic Gaucher disease, the CNS is an elusive treatment area highlighted in MPS and neurological progression persists despite ERT (Noh and Lee 2014).

In infantile Pompe disease (IPD), ERT with alglucosidase alfa has changed the natural history of the disease. Improvements in cardiac and motor function have been observed in long-term survivors of IPD. However a new emerging phenotype is evident due to the longer term survival of these patients which includes proximal and distal myopathy, sensorineural hearing loss, risk for arrhythmias, hypernasal speech, dysphagia (with risk for aspiration), ptosis, and osteopenia (Jones et al. 2010; Nicolino et al. 2009; Yanovitch et al. 2010; Prater et al. 2012) again portraying lack of effective penetration/activity of ERT in critical target tissues. A study of long-term ERT in IPD patients indicates that skeletal muscle damage persists in patients despite ERT, including those started within the first month of life (albeit to a lesser extent than in patients who start ERT at an older age) (Prater et al. 2013). There is also involvement of the anterior horn cells, and other CNS manifestations

including delayed processing speed that is noted in long term IPD survivors (Spiridigliozzi et al. 2013). Residual deficits in late onset Pompe disease treated with ERT include respiratory insufficiency and difficulty walking and/or climbing (Kobayashi et al. 2010; Strothotte et al. 2010).

Such persistent debilitating complications seen in LSDs treated with ERT exemplify how ERT, although a monumental step towards improving the quality of life for patients, is not optimal and certainly not curative.

Present efforts towards bridging the gap between currently available therapies and a cure for LSDs are best viewed through the lens of our collective experience with Pompe disease. About 9 years after ERT was established for Gaucher disease, clinical trials for ERT in Pompe disease unfurled with very promising results. The intravenous administration of alglucosidase alfa (GAA) for Pompe disease blazed the trail as proof of concept for ERT use in neuromuscular disorders. Pompe disease, a true disease spectrum, presents broadly as an infantile and adult onset form (Kishnani et al. 2012). Across the continuum of Pompe disease, the enzyme GAA is partially or completely deficient, leading to the accumulation of glycogen in many tissues, especially the cardiac, skeletal, and smooth muscles. Since alglucosidase alfa's approval by the FDA in 2006, many benefits, challenges, and underlying issues have been highlighted. ERT dramatically transformed the prognosis for Pompe disease: the infantile onset form is no longer fatal within the first year of life and the adult onset form improves or stabilizes instead of worsening in many of the patients treated. With the adult onset patients living longer and the infantile onset patients surviving past 1 year, the natural history of the disease spectrum is becoming better understood and increasingly useful to parallel with other GSDs and LSDs. Although progress with ERT is notable in Pompe disease, long-term issues stemming from delayed therapy initiation and/or poor ERT delivery to certain sanctuary sites are becoming evident. While enhanced newborn screening programs, which aim to target neonates and infants prior to symptom onset and enable early treatment initiation (Liao et al. 2014), have reduced delayed administration of ERT, residual motor deficits, which can cause hypotonia and fatigue, are still noted in IPD (Chien et al. 2013).

Pompe disease serves as a useful treatment model for several reasons, including the applicability of ERT across the whole disease continuum spanning from infantile (severe) to adult (less severe) onset and the multi-systemic nature of Pompe disease. The efficacy of ERT can be tested on patients of all ages and its effects on different disease manifestations, *i.e.,* cardiac, skeletal muscle involvement and respiratory involvement in infantile and late onset disease, can be compared. The extent of musculoskeletal involvement is also monitored across the disease spectrum, particularly in relation to the effect of ERT on its progression and patient's age at start of therapy. The infantile presentation, in particular, provides insight into the long-term effects of treatment, as well as on the importance of early treatment. Pompe disease especially serves as a beneficial model due to the rapidity of disease progression: IPD left untreated proves fatal by 1 year of age and the clinical effects of ERT and of immune responses to ERT are readily apparent. The availability of a mouse model for Pompe disease is another strong point in this disease. Animal testing enables better understanding of cellular mechanisms of Pompe disease as well as the effects of potential treatments on said mechanisms.

Although ERT in Pompe disease has proven to be very successful in many regards, several limitations exist: the efficiency of ERT distribution and uptake in the extensive muscular system, inability of ERT to cross the blood–brain barrier, pathological preconditions, defective cellular machinery, and immune responses to ERT. The biggest challenge remains the enormity of the target organ, muscle. ERT is required in high doses, 30–100 times greater than in other LSDs, to attempt to saturate muscle, which makes up 40 % of body mass (Desnick 2004). The heterogeneity of the muscular system and the muscle fiber type may also contribute to the variable response of different tissues. It has been shown that cardiac muscle responds much better to ERT than skeletal muscle, one theory being that because skeletal muscle has both an endothelial barrier and endomysium, an exogenously introduced enzyme may be deterred. Another theory for suboptimal skeletal muscle response lies in the density of cation-independent mannose 6-phosphate (M6P) receptors essential for the binding and uptake of ERT into the muscle and trafficking to lysosomes. Cardiac muscle tends to have a much higher density of M6P receptors than does skeletal muscle, corresponding with the level of response to ERT (Raben et al. 2003; Winkel et al. 2004; Zhu et al. 2004). M6P receptors recognize ERT molecules via their expression of M6P residues, which target them for transport to the lysosome and are therefore key in delivering exogenous enzyme to the lysosome for glycogen degradation.

Biobetters, in which the ERT is engineered to express high levels of bis-mannose 6-P residues, could substantially improve uptake of ERT into skeletal muscle cells, as could enhancing expression of M6P receptors on skeletal muscle cells. Similar to the inhibitory effects of the endomysium and endothelial linings surrounding skeletal muscle tissue, ERT also cannot cross the blood–brain barrier to break down glycogen accumulation in the CNS. This section of the book includes a chapter discussing the means to address CNS penetration of therapeutic proteins.

A number of pathological factors in Pompe disease also govern response to ERT. The degree of muscular and lysosomal damage present at the time of ERT administration affects outcome: patients with little damage fare better (Kishnani et al. 2007). This finding highlights the importance of accurate and early diagnostic techniques, i.e., newborn screening, which in turn would lead to an early treatment (Chien et al. 2011; Burton 2012; Shigeto et al. 2011). The muscle fiber type also contributes to outcome. GAA enzyme breaks down less glycogen in “fast twitch” type II muscle fibers, whereas “slow twitch” type I muscle fibers tend to undergo significant glycogen clearance by ERT. In one study, muscle biopsies of eight infantile onset patients who were on ERT showed varied reduction of glycogen accumulation (Thurberg et al. 2006). Those patients who had lower glycogen storage, milder cellular damage, more type I muscle fibers, and earlier initiation of ERT typically exhibited better clinical outcome.

Defective autophagy, contributing to disease pathology, is being noted in patients treated with ERT. Lysosomal storage of substrate has been found to impair proper autophagic function, which, in both Pompe disease patients and in GAA knock out mice, correlated to muscle weakness, buildup of dysfunctional mitochondria, and muscle atrophy (Shea and Raben 2009; Raben et al. 2012; Nascimbeni et al. 2012).